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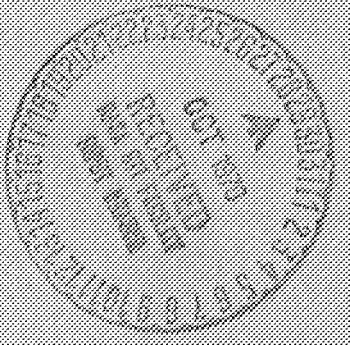

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Adipose tissue in altered lipid metabolism of rats exposed to centrifugation stress

D. D. FELLER, E. D. NEVILLE, AND
K. S. TALARICO
Environmental Biology Division, Ames Research Center, National Aeronautics and Space Administration, Moffett Field, California

FELLER, D. D., E. D. NEVILLE, AND K. S. TALARICO. *Adipose tissue in altered lipid metabolism of rats exposed to centrifugation stress.* Am. J. Physiol. 214(6): 1434–1437. 1968.—Male Sprague-Dawley rats, 9 weeks of age, were centrifuged at 47. G for periods from 0.5 hr to 21 days. The concentration of serum glucose, free fatty acids, triglycerides, and cholesterol was measured periodically. The conversion of acetate-2-^{14}C into $^{14}CO_2$ and fatty acids in slices of epididymal fat pads and its fatty acid content was studied. Significant increases in newly formed fatty acids were found in adipose tissue excised from rats exposed to centrifugation for periods of 24 hr or less. Serum glucose, free fatty acids, and triglycerides increased during this period. As the exposure continued beyond 3 days, serum glucose fell below control values, depot fat stores decreased, and the conversion of acetate-2-^{14}C to fatty acids in adipose tissue from stressed rats returned to control values. No significant change in acetate-^{14}C oxidation to $^{14}CO_2$ by adipose tissue was noted. It is concluded that adipose tissue plays an important role in contributing to body energy during the acute phase of centrifugation stress in rats.

adipose tissue lipogenesis; fat stores; serum lipids

As reported earlier (5) an increased conversion of radioactive acetate to fatty acids was observed in the liver of rats exposed to 4.7 G. This conversion is dependent on the age of the animal and duration of exposure to acceleration. Further study (6) indicated several metabolic changes as the exposure was continued to 24 hr. Plasma glucose and plasma corticosterone curves were bimodal, showing an early maximum during the first 3 hr of exposure and again rising after 5 hr through the 24-hr study. The decrease in liver fat that followed the initial increase was accompanied by an increase in hepatic fatty acid synthesis. It was apparent from these findings that dramatic changes in mobilization of fat were occurring as a result of exposing rats to simulated increased gravity. Thus, as an extension of the work described above, it became of interest to study the changes of lipid content in adipose tissue as exposures were extended for periods of 3 weeks, and to study the conversion of acetate to fatty acids and the oxidation of acetate to CO_2 in this tissue. For a more complete understanding of the observed effects, changes in the various lipid fractions of serum were also of interest. It is these aspects of the metabolic alteration to centrifugation stress that are the subject of the present paper.

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METHODS

Male Sprague-Dawley rats (Simonsen Laboratory, Gilroy, Calif.) were exposed to a 4.7-G environment on a 4.5-ft radius centrifuge (6) for periods ranging from 0.5 hr to 3 weeks; noncentrifuged rats served as controls. The rats were selected so that at time of sacrifice, the mean age of the experimental and control groups was approximately the same. The experimental and the control groups consisted of six rats each for every time interval studied so that the data could be analyzed statistically by two methods: the Student *t* test and analysis of variance (11). From weaning, rats were fed freshly prepared Simonsen's maintenance diet containing 6–8% fat. Since rats ingest lesser amounts of food when placed on the centrifuge (10), the control animals were pair fed. Rat weights are recorded (Table 1) at the start and end of centrifugation. Although the average daily amount of food the rats on the centrifuge consumed could be measured, the manner in which they consumed this food was not determined. Therefore, although both the centrifuged and control animals ate the same quantity of food daily, differences between them in the manner of eating are possible. Both groups of rats were deprived of food 22–24 hr prior to sacrifice and, consequently, for animals centrifuged 24 hr or less only the final rat weight was measured. Blood was withdrawn and animals were killed for removal of tissue between 9:00 and 11:00 AM each experiment day.

Analytical procedures. Immediately after the rats were removed from the centrifuge, both the experimental rats and the controls were anesthetized with sodium pentobarbital (40 mg/kg body wt, ip), and blood for serum was withdrawn by syringe from the lower aorta. The

TABLE 1. *Effect of exposure time on fatty acid content and conversion of acetate-2-^{14}C to fatty acids and CO_2 in adipose tissue*

Exposure Time	Body Wt, g				Adipose Tissue Fatty Acids, %		% of Conversion per g			
	Control		Centrifuged		Control	Centrifuged	To CO_2		To fatty acids	
	Initial	Final	Initial	Final			Control	Centrifuged	Control	Centrifuged
0.5 hr		242±2		240±3	70.1±2.6	68.8±3.4	6.3±0.4	7.0±0.9	4.6±0.8	3.6±0.2
1		202±4		204±6	70.6±2.1	68.2±1.1	9.2±0.6	10.3±0.9	7.0±0.8	14.9±0.5*
3		221±1		216±2	69.7±2.0	65.0±4.1	7.4±0.4	9.1±0.4	5.2±0.5	4.4±0.7
4		204±3		202±4	68.0±2.2	68.1±1.4	9.2±0.5	9.2±0.4	7.8±1.0	12.8±1.3*
5		258±3		254±4	71.0±1.4	74.3±1.0	6.7±0.8	6.1±0.5	6.8±0.6	15.1±1.2*
8		259±3		255±1	70.7±1.3	71.0±1.8	6.8±0.8	8.0±0.2	6.8±0.6	15.6±2.4*
12		204±4		201±7	70.9±2.1	70.7±1.2	9.2±0.7	10.2±0.6	7.0±0.8	20.1±1.3*
16		232±3		228±1	68.7±2.4	62.9±2.0	6.8±1.2	7.8±0.4	5.6±0.4	10.4±0.8*
24		219±1		217±3	69.0±1.9	65.8±4.2	7.7±0.4	7.9±1.0	5.4±0.4	9.4±0.8*
3 days	212±2	194±3	211±2	187±2	66.1±2.4	68.6±2.0	7.0±0.7	5.3±1.1	3.5±0.3	3.8±0.4
7	213±2	199±2	213±2	196±6	60.8±2.5	66.8±1.4	8.6±0.7	6.7±0.7	7.5±1.8	5.6±0.9
14	183±2	161±4	181±3	156±2	52.4±5.4	37.7±5.3	14.0±1.4	17.0±1.1	6.0±0.8	6.6±1.1
21	182±3	174±3	177±1	167±3	64.1±1.6	50.9±4.4	15.1±0.9	14.3±1.8	3.7±0.5	5.0±0.7

1.0 g of epididymal adipose tissue obtained from 22- to 24-hr fasted rats exposed to 4.7 G for 0.5 hr to 21 days was incubated for 3 hr at 37.5 C in 10.0 ml of buffer. Each value represents the mean ± SE of 6 rats. Body weights are those at the start and at the end of 3 days or more of centrifugation. * Mean from centrifuged group found to be significant ($P < 0.05$) from the non-centrifuged pair-fed control group as judged by the t test.

serum was frozen until analyzed 2–3 days later. One gram of epididymal adipose tissue was cut with a scissors and incubated at pH 7.4 for 3 hr in 10 ml Krebs bicarbonate buffer containing 0.01 M succinate, 0.011 M glucose, and 10 μc of 0.001 M sodium acetate-2-^{14}C as described earlier (5). The incubation reactions were terminated by adding 0.2 ml 10 N H_2SO_4 to the medium. The CO_2 liberated was absorbed with 1 ml 2.5 N NaOH added to a removable plastic CO_2 collection well which was inserted prior to sealing the system (9). Fatty acids were saponified by heating the tissues overnight in a solution of alcoholic-KOH on a steam bath. The non-saponifiable tissue lipids were removed with three hexane extractions and discarded. Tissue fatty acids were extracted with aliquots of hexane after acidification at 4 C with 6 N HCl. The total tissue fatty acid and ^{14}C content of fatty acids and CO_2 was determined (5).

Serum (1 ml) was extracted with 12 ml of chloroform-methanol (2:1, v:v) as described by Jover (8). Aliquots of the chloroform phase were taken to determine serum total lipid (2), triglyceride (8), and total cholesterol (1). Serum free fatty acids (FFA) were determined on 1 ml of serum by the method of Trout (12). Serum glucose was determined on 0.5 ml of serum by the glucose oxidase method (3).

RESULTS

Serum glucose rose to levels significantly higher than those of the controls after 0.5 hr of centrifugation and remained so for the 1st day (Fig. 1). Peak values as high as 230 mg/100 ml were noted while control values ranged from 90 to 110 mg/100 ml. At 3 and 7 days of centrifugation, serum glucose levels from centrifuged rats were not significantly different from those of noncentrifuged controls. At the 2- to 3-week period, the serum glucose level of the centrifuged rats was 75 mg/100 ml which was significantly lower than the 100 mg/100 ml level of the control rats.

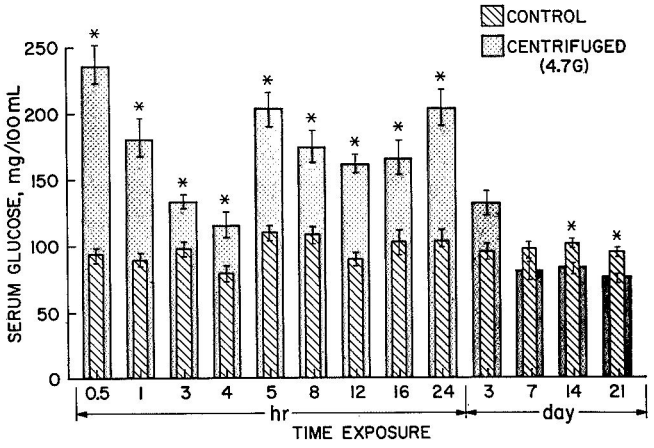

FIG. 1. Effect of exposure time on serum glucose of 22- to 24-hr fasted, 9-week-old rats exposed to 4.7 G. Each value represents mean ± SE of 6 animals. Asterisk indicates that the mean of the centrifuge group is significantly ($P < 0.05$) different from the mean of pair-fed control group.

The changes that occurred in serum FFA are shown in Fig. 2. An increase in FFA was noted in serum from rats exposed to 4.7 G for 0.5 hr. The FFA levels of the experimental rats remained significantly higher than the control values for seven time periods studied during the first 24 hr of exposure. During this 24-hr period, the greatest net increase found was 250 μEq/liter at the 24-hr time period. The highest value observed was 760 μEq/liter for the experimental group at 8 hr compared to 608 μEq/liter for the controls. FFA values were not significantly elevated after 3 days of centrifugation except at the 1st week when a significant increase over the control value was observed.

Serum triglyceride levels rose significantly higher than control values during the 1st hr of centrifugation as shown in Fig. 3. After 1 hr of exposure to acceleration stress, the serum triglyceride from the experimental group peaked at 107 mg/100 ml compared to control value of 70 mg/100 ml. At the 3rd hr, the triglycerides returned to normal serum values.

Not shown in figures were the results of changes in total serum lipid and serum cholesterol. The only notable change observed in these two blood components occurred after 3 and 7 days of centrifugation when serum cholesterol from experimental rats rose from a control value of 62 mg/100 ml to 78 mg/100 ml. The difference was significant as judged by the t test.

The changes observed in the chemistry and metabolism of adipose tissue in rats exposed to 4.7 G by centrifugation are shown in Table 1. Table 1 also shows the changes in body weights of noncentrifuged rats on a pair-fed diet for 3–21 days and of centrifuged rats exposed for these periods of time. The weight loss for both groups (experimental and pair-fed controls) was of the same order of magnitude. The rats did not gain weight and were from 6 to 16% lighter at the end of the experimental period than at the beginning. The amount of fatty acids contained in the epididymal fat pad was reduced. The adipose tissue pad of the noncentrifuged control rat started with a composition of 70% of its weight as fatty acids and after 14 days of pair feeding, 52% of its composition was fatty acids. For centrifuged rats the composition of fatty acids was reduced to 38%. The loss of fat from the pad led to a decrease in the total amount of adipose tissue in the rats centrifuged for these longer periods of time. The weight of the animal became somewhat stable and the loss of fatty acid composition from the adipose tissue was lessened at 21 days as the animals consumed normal amounts of food (10).

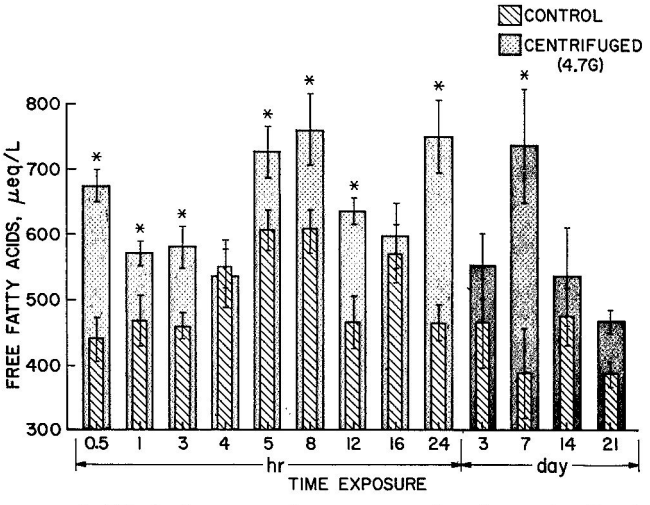

FIG. 2. Effect of exposure time on serum free fatty acids (FFA) of 22- to 24-hr fasted 9-week-old rats exposed to 4.7 G. Each value represents mean ± SE of 6 animals. Asterisk indicates that the mean of the centrifuge group is significantly ($P < 0.05$) different from the mean of pair-fed control group.

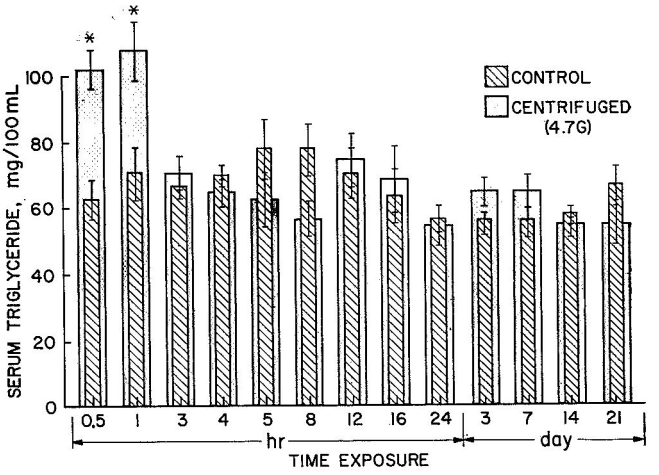

FIG. 3. Effect of exposure time on serum triglycerides of 22- to 24-hr fasted, 9-week-old rats exposed to 4.7 G. Each value represents mean ± SE of 6 animals. Asterisk indicates that the mean of the centrifuge group is significantly ($P < 0.05$) different from the mean of the pair-fed control group.

Fatty acid synthesis as measured by conversion of acetate-2-^{14}C to fatty acid per gram of adipose tissue was significantly increased during the 4- to 24-hr period and also at the 1-hr period. The greatest increase was observed at the 12-hr period when the tissue from centrifuged rats converted 20.1% of added acetate-^{14}C to fatty acids per gram tissue, while the controls converted a mean of 7.0%.

No significant changes were observed in the oxidation of acetate-2-^{14}C to CO_2 by tissue from centrifuged rats compared to noncentrifuged controls. An increase in oxidation was noted for both groups at 14 and 21 days. This corresponds to the time when the rats consumed amounts of food equal to the daily rations of noncentrifuged, ad lib., control rats of the same age (10).

DISCUSSION

The changes in lipid metabolism of stressed rats can best be described by the responses that were observed as the duration of exposure to centrifugation was lengthened. During the first hours of centrifugation, there was an immediate rise in the blood concentration of serum glucose, FFA, and triglycerides. These changes occurred before any changes were noted in fatty acid content of adipose tissue, in oxidation of acetate to CO_2 by adipose tissue, in rat body weight, and before a sustained change was noted in conversion of acetate-2-^{14}C to fatty acids by adipose.

During the 1st day of centrifugation, serum triglyceride levels returned to control values while serum glucose and FFA concentrations remained high. The amount of acetate-2-^{14}C converted to fatty acids by adipose tissue remained significantly elevated throughout the 4- to 24-hr period. During this acute response to centrifugation stress, no appreciable effect was noted in the oxidation of acetate by the tissue, nor was any change noted in body weight or lipid content of adipose tissue. It is during this

phase that the stress-induced increase in conversion of acetate to fatty acids most likely results from the interplay between adrenal corticosterone, insulin, and circulating glucose (4, 6). The response is abolished in tissues obtained from adrenalectomized or alloxanized rats and restored only with administration of glucose in the case of the former or by treatment with insulin in the case of the latter (4). The hypothesis that increased lipogenesis in tissues from stressed rats results from high circulating glucose and insulin is supported by the results found here and in an earlier report (6).

Finally, a compensatory phase (3–21 days) of the experiment was reached. During this period a loss of body weight was noted; a reduction in amount of fatty acids in adipose tissue also occurred, although some recovery of this loss was observed by the 3rd week of centrifugation. In general, the changes observed during the 1st day had disappeared during the final phase. The decrease in serum glucose below control values after 2 and 3 weeks of centrifugation, the greater loss of fatty acids in adipose tissue of centrifuged rats, and the single observation of increase in serum FFA at the 7th day occurred at a time when the amounts of food consumed by the animals increased to normal levels (10) and when a greater than normal expenditure of body energy was expended by increased muscular activity required to overcome the physical aspects imposed by centrifugation.

Jordan and co-workers reported recently (7) on altered metabolism of radioactive acetate in vivo in rats exposed for 36 days to a high-oxygen environment at reduced pressure. This environment caused no reduction in body weight of the animals but, since the expiration of $^{14}CO_2$ was reduced as was the incorporation of radioactivity into tissue lipids, the environment did reduce metabolic activity. In contrast, the studies reported here show an increase in the conversion of acetate-2-^{14}C into fatty acids with no change in the incorporation of acetate-2-^{14}C into $^{14}CO_2$. Thus, this response of adipose tissue lipogenesis to centrifugation stress reflects a specific effect on fat metabolism and not a general change in acetate metabolism.

The results presented here show that adipose tissue plays an important role in contributing energy by releasing lipids during the acute phase of centrifugation stress in rats. When the rats are not gaining weight and when adipose tissue is yielding lipids to serum, adipose tissue responds to the stress by synthesizing greater than normal amounts of fatty acids. For longer exposure after the stimulus for increased insulin secretion is lost, the accelerated loss of fat from peripheral stores is no longer made good by enhanced fatty acid synthesis in the adipose tissue, as the result of which the peripheral fat stores become depleted. Furthermore, since this depletion of the fat stores occurs under circumstances in which circulating free fatty acids are no longer elevated and in which the animals are pair-fed isocalorically with their controls, then it must follow that the energy expenditure of the centrifuged group is high in relationship to the food intake and that their metabolic adaptation to the increased sustained work load is as yet imperfect.

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